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TITLE: "Enhancing Peripheral Nerve Regeneration with a Novel Drug-Delivering Nerve Conduit"

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project is directed at improving the rate of nerve regeneration, through the development and testing of a novel nerve conduit. This project explores the potential of combing a nerve conduit that will act as an axon guide for the regenerating nerve and a drug delivery device to deliver nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) for at least 30 days to improve rate of nerve regeneration. We have successfully manufactured a dual chamber device, as well as further validated a diffusion model that can be used to design the nerve conduit a priori. That is by knowing what concentration of growth factor we want to deliver and for what duration we can then determine the approximate reservoir volume, diffusion hole, and growth factor concentration that need to be established to meet these design criterion. We have further determined the concentration of NGF and GDNF that need to be delivered simultaneously to result in optimal nerve growth. Finally, we have acquired a breeding pair of mice that express GFP in their peripheral nerves, begun expanding the colony, and have confirmed that the progeny express GFP in their peripheral nerves. Over the next year we will further characterize the release kinetics and bioactivity of NGF and GDNF released from the device and evaluate the in vivo efficacy of our novel nerve conduit.

15. SUBJECT TERMS

Nothing listed

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1. INTRODUCTION:

This project is directed at improving the rate of nerve regeneration, through the development and testing of a novel nerve conduit. Our technology is a fusion between a nerve conduit and a biodegradable drug reservoir. Combat gear for the modern day warrior has greatly improved protection for the head and body, but limbs are still highly exposed to injury. Subsequently, the most frequent combat nerve injuries are in the upper and lower extremities. Patients often suffer from life-long loss or functional disturbances mediated by the injured nerve, which can severely diminish their quality of life. Autologous nerve grafts serve as the state-of-the-art but numerous challenges associated with this approach results in functional benefits to only 40-50% of patients with the additional consequence of nerve-graft donor site morbidity.

This project will explore the use of combing a nerve conduit that will act as an axon guide for the regenerating nerve and a drug delivery device to deliver nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) for at least 30 days to improve the nerve regeneration. The use of PLGA or collagen has been tried previously. These devices typically focus on loading the drug in hydrogels or embedding the drug within the conduit. The shortcomings of current devices in terms of burst effect, non-uniform dosage, and uneven drug delivery, necessitates a new approach to deliver drug for nerve regeneration. The currently proposed design is advantageous, as it will allow for controlled drug release that can be tailored made with the ability to vary the concentration, duration, and rate of release of multiple drugs.

2. KEYWORDS:

Nerve regeneration, peripheral nerve regeneration, nerve conduits, autograft, drug delivery device, nerve growth factor, glial cell line-derived neutrophic factor, polytetrafluoroethylene, nerve grafts, axon growth, dorsal root ganglion cells, axon density, motor neuron, sensory neuron

3. ACCOMPLISHMENTS:

3.1 What were the major goals of the project?

Specific Aim 1 -- To optimize release kinetics of NGF and GDNF in vitro using our novel drug delivery conduit.

Tasks/Subtasks:
1. Manufacture Devices for use in 15mm nerve gap .... (Gale,)(0-10 months)
    a. Optimize PGLA ratios .... (Gale/Terry)(0-4months)
    b. Optimize nanoporous membrane dimensions................... (Gale)(2-6months)
    c. Optimize reservoir dimensions... (Gale)(4-8months)
    d. Manufacture and assemble components .... (Gale)(6-10months)

Progress: We have completed these tasks. We have done a device redesign this year based on our results from another DOD funded project (PR121391). The PLGA device did not maintain its structure in the early healing phase (21 days post implantation), and nerve regeneration was not optimal. Thus, we redesigned the conduit and re-completed the tasks of Aims 1 (see section 3.2.a for more detailed specific accomplishments).

Specific Aim 2 -- To evaluate the effectiveness of the conduit-drug delivery device at releasing bioactive concentrations of NGF and GDNF sufficient to enhance axon growth in dorsal root ganglion (DRG) cell culture

Tasks/Subtasks:
1. In Vitro NGF/GNDF release kinetics experiments.......................... (Gale, Agarwal)
    (11-18months)
2. ELISA detection of NGF/GDNF.. (Gale, Agarwal)
    (11-18months)
3. Axonal growth of DRGs.. (Terry, Shea)
    (11-18months)

Progress: We have started these tasks as planned. Initial release kinetics from our new device are preforming as our model predicted and are within the range that we had previously determined to be beneficial to nerve (DRG) growth. We have established the bioactivity of released NGF and GDNF from our previous device design but are repeating for our new design to confirm our earlier results. We had previously optimized the ELISA detection of NGF and GDNF and are in the process of completing evaluation of the 30 release of NGF, GDNF, and combined release of NGF and GDNF. We have all protocols established for the evaluation of bioactivity of the released proteins using DRGs. Due the redesign of our device, we are slightly behind schedule with the completion of Aim 2 but will finish the aim within the next couple of months. We are approximately 40% finished with the tasks of Aim 2.

Specific Aim 3 -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a nerve gap in a mouse sciatic nerve model.

1. IACUC approval, obtain N=160 animals .... (Agarwal, Shea)
    (19-20 months)
2. Implant Device ½ devices (Mix 30-90 day groups) .... (Agarwal, Shea)
    (21-25months)
        a. ELISA for NGF/GDNF detection of day 30 animals........... (Gale)
            (24-29 months)
        b. Walking Track.. (Agarwal, Shea)
            (21-28months)
        c. Histology... (Agarwal, Shea)
            (22-32months)
            (H&E, immunohistochemistry, retrograde labeling, muscle histology)
        d. Electrophysiology .... (Clark)
            (24-30months)
3. Implant Device ½ devices (Mix 30-90 day groups) .... (Agarwal, Shea)
    (26-30months)
        a. ELISA for NGF/GDNF detection of day 30 animals........... (Gale)
            (29-33 months)
        b. Walking Track.. (Agarwal, Shea)
            (26-33months)
        c. Histology... (Agarwal, Shea)
            (27-34months)
            (H&E, immunohistochemistry, retrograde labeling, muscle histology)
        d. Electrophysiology .... (Clark)
            (29-34months)
4. Data Compiling/Analysis and Manuscript Preparation .... (Agarwal, Gale,

Shea, Clark, Terry) (30-36months)

Progress: We have obtained IACUC and ACURO approval. We have purchased a breading pair of mice and have commenced with colony expansion. Our colony will be sufficiently expanded to begin implantation, once the device release kinetics have been further validated (Aim 2). We have optimized retrograde labeling, osmium tetroxide staining, and neuromuscular junction staining. We have completed 10% of tasks for Specific Aim 3.   We are behind schedule as a result of the device design but everything is set in place to begin the in vivo experiments once the device release kinetics are finished.

3.2 What was accomplished under these goals?

Major Activities
Tasks/Subtasks:
Task 1. Manufacture Devices for use in nerve gap
      a. Optimize PGLA ratios
      b. Optimize nanoporous membrane dimensions
      c. Optimize reservoir dimensions
      d. Manufacture and assemble components
Progress: We have completed these tasks as planned.

Task 2. In Vitro NGF/GNDF release kinetics experiments
Task 3. ELISA detection of NGF/GDNF
Task 4. Axonal growth of DRGs
Progress: We have completed about 40% of specific Aim 2 tasks. We are currently behind schedule but the new device design release kinetics are currently underway. We have preliminary data with the release of fluorescently labeled dextran that indicate the new device is sealed and able to release therapeutics in a controlled manner over an extended time frame. We are confirming these results with NGF and GDNF.

Task 5. IACUC approval, obtain N=160
Task 6. Implant Device ½ devices (Mix 30-90 day groups)
      a. ELISA for NGF/GDNF detection of day 30 animals
      b. Walking Track
      c. Histology   (H&E, immunohistochemistry, retrograde labeling, muscle histology)
      d. Electrophysiology
Task 7. Implant Device ½ devices (Mix 30-90 day groups)
      a. ELISA for NGF/GDNF detection of day 30 animals
      b. Walking Track
      c. Histology   (H&E, immunohistochemistry, retrograde labeling, muscle histology)
      d. Electrophysiology
Task 8. Data Compiling/Analysis and Manuscript Preparation
Progress: We have obtained the IACUC approval. We have obtained the breeding pair and are in the process of expanding the colony so that we can commence with the surgeries once the release kinetics are completed. We have completed 10% of tasks for Specific Aim 3.

Specific Objectives
Specific Aim 1 -- To optimize release kinetics of NGF and GDNF in vitro using our novel drug delivery conduit.

Specific Aim 2 -- To evaluate the effectiveness of the conduit-drug delivery device at releasing bioactive concentrations of NGF and GDNF sufficient to enhance axon growth in dorsal root ganglion (DRG) cell culture
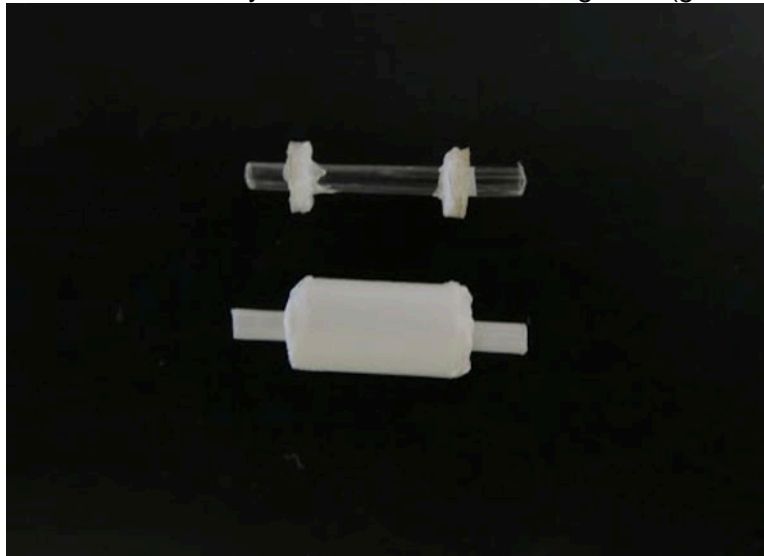
Specific Aim 3 -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a nerve gap in a mouse sciatic nerve model.

3.2 Significant Results

3.2.a Aim 1 Device Fabrication.

The design criterion for our nerve guide is to release both NGF and GDNF locally while also supplying a guidance tube to enhance peripheral nerve regeneration. We have manufactured a dual chamber nerve guide

PGLA Nerve Conduit*:* Our initial device design was made with a 75 to 25 poly-lactic-acid to poly-co-glycolic-acid copolymer ratio (PLGA). The device consisted on an outer PLGA tube, end caps defining the boundaries of the reservoir, inner conduit, and a diffusion hole on the inner conduit that allows for the diffusion of therapeutics from the reservoir to canal of the inner conduit (Figure 1). Our device design allows for easy modification of drug choice, concentration, or released volume by altering basic design parameters such as reservoir volume or diffusion hole size. In the process of developing our device, we have created a mathematical model that allows us to estimate how changing the volume of the reservoir, concentration of drug, and hole size alter release kinetics (Figure 2; orange line). We were able to accurately predict the release of fluorescently labeled dextran, with a similar diffusion coefficient to NGF, over a period of approximately 40 days. The PLGA molecular weight and combination (75:25; PLA:PGA) was chosen such that the drug diffuses only through the diffusion area and does not permeate through the walls, as demonstrated by the sealed devices in Figure 3 (green line).

Figure 1. (Top) Inner conduit with two end caps defining the outer boundary of the reservoir. (Bottom) Completed PLGA device with inner and outer tube, as well as a diffusion hole on the inner conduit that enables the release of NGF from the reservoir into the inner chamber.
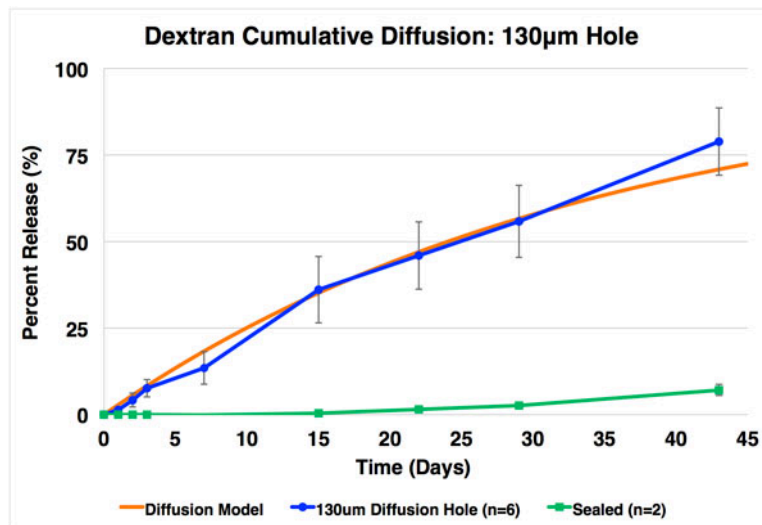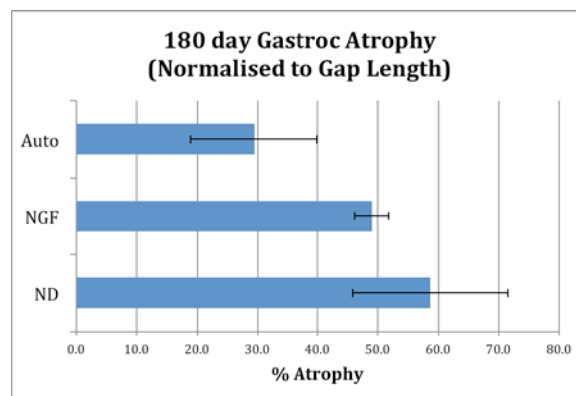
Figure 2. Fluorescently labeled dextran (blue) was released from our PLGA nerve conduit device for approximately 40 days, with data comparing well to our model predication (orange). Additionally, the sealed device (green) did not release dextran suggesting all drug was released through the diffusion hole.

We implanted the single chamber device that released NGF in a rat sciatic nerve gap model, under another DOD grant (PR121391). When we harvested the nerve guide at 21 days we observed that the device had collapsed resulting in the inner conduit diameter being considerably smaller than designed (Figure 3). At six months post implantation we further observed that the group with the NGF nerve guide device had greater muscle atrophy compared with the autograft group but slightly lower atrophy compared to the no drug group (Figure 4). The combined in vitro and vivo resulted suggested two important findings: 1) We were able to release fluorescently labeled dextran from our device for 40 days and in manner in accordance with our diffusion model, therefore the general concept of our device is sound. That is the reservoir nerve conduit device can release growth factors for extended periods of time in a controlled and predicable manner. 2) The structure of the device upon harvest at 21 days and the limited muscle regeneration in the group receiving the nerve guide device suggests that the PLGA material we initially chose was not the ideal material. We therefore wanted to choose a material that can maintain its structure for the duration of the in vivo experiment and to further validate the device design which will improve nerve regeneration in the setting of peripheral nerve repair. Thus, for subsequent device design we are utilizing polytetrafluoroethylene (PTFE), which is more stable in vivo than PLGA.
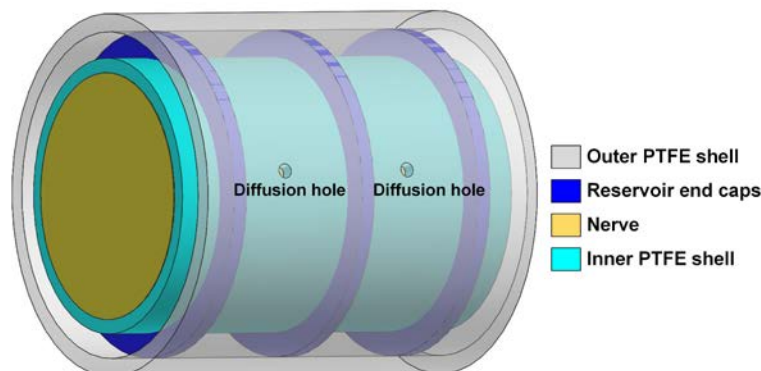
Figure 3. The PLGA nerve conduit device after 21 days implantation in a rat sciatic nerve gap model. The device compressed resulting in a smaller inner conduit diameter. It is also possible that leaks could have formed within the reservoir as a result of the deformation.

Figure 4. As a result of degradation of the PLGA the nerve gap was not consistent between animals at the time of harvest. Thus atrophy was normalized to the distance between the sutures (proximal and distal) at harvest.

PTFE Nerve Conduit: For the present proposal our goal was to have a dual chamber nerve guide that can release growth factors for at least 30 days. The general concept of the device is illustrated in Figure 5 and utilizes a similar approach as the PLGA device. That is the major components of the device are the same: inner conduit, outer conduit, and diffusion hole. The only difference is the materials utilized to manufacture the device.
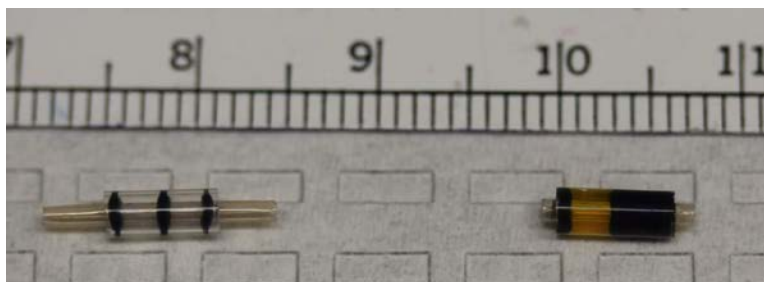

Figure 5. Diagram of our polytetrafluoroethylene (PTFE) nerve conduit device. Drug (NGF and GDNF) loaded in the reservoir between the outer and inner conduit will diffuse through the

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diffusion hole and enter the inner tube, contacting nerve stumps and stimulating axon growth. The inner tube is attached to the two nerve stumps via suture and provides a guide for the regenerating nerve.

Since the device will be implanted into mice for the present set of experiments we have reduced the size of the device from our single chamber device used in rats. Our device parameters are that NGF and GDNF need to be released from the device for at least 30 days and the inner conduit needs to be 10mm long. Based on our diffusion model the reservoir needs to be loaded with approximately 5µL of growth factor and the diffusion hole needs to roughly 120µm in diameter to release NGF and GDNF for 30 days.   Additionally, our in vitro release results (see Release Kinetics 3.2.b) suggest that when released simultaneously we need to release both NGF and GDNF at a concentration range of 0.1-10.0 ng/mL to achieve the greatest enhancement of nerve growth. Therefore, we will need to load the reservoir with NGF and GDNF at a concentration 50mg/mL to achieve the proposed release parameters.

The devices were assembled using PTFE tubes and nitrile rubber based o-rings. PTFE tubes of diameter 1 mm were used as inner conduits (Figure 6). A laser was utilized to create diffusion holes in the inner conduit at 2.75 mm from both ends, so as to be in the middle of each chamber. O-rings of ID 1 mm and a cross section of 0.5 mm were slid on the inner tube and placed 1 mm from the end, another o-ring was placed as a chamber divider in the middle. PTFE tube of ID 1.9 mm was used as the outer conduit and slid over the o-rings to finish the device assembly. The reservoir chambers were filled using a 33 gauge needle.
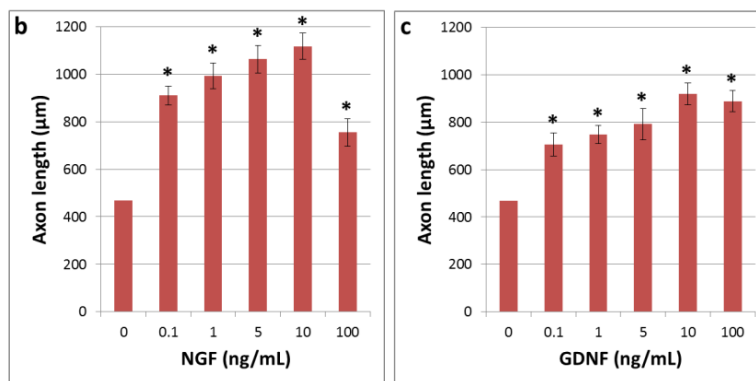

Figure 6. (Left) Photograph of an assembled PTFE device with inner conduit, outer conduit, and reservoir. (Right) Photograph of a PTFE nerve conduit device loaded with fluorescein labeled dextran and ruby red labeled dextran.

3.2.b Aim 2 Release Kinetics/DRG Growth: We have completed an investigation into the optimal dosage of NGF and GDNF that needs to be released from the device to enhance nerve regeneration. Specifically fertilized chicken eggs were incubated at ~39°C under 100% relative humidity for 12 days. Dorsal root ganglions (DGRs) were dissected from the embryos under a stereomicroscope, separated from connective tissue, and cultured in 24-well plates coated with laminin (1 mg/ml). DRGs were plated at a density of one per well, and growth factors were added as specified below. DRG cultures were maintained in a humid atmosphere at 37°C and 5% CO_2 for 72 h. For each experimental condition including control, four DRGs of similar size were used and the experiments were repeated 3-4 times. In the single growth factor experiments, the DRGs were treated with increasing concentrations of either NGF (0.1, 1, 5, 10, 100 ng/ml) or GDNF (0.1, 1, 5, 10, 100 ng/ml). For control, DRG-explants were incubated in cell culture media matrix (DMEM+10% FBS+1% Antimyotic) without any growth factor or drug. In the experiments using combined growth factors (NGF/ GDNF), the DRGs were treated with random dosages chosen using the statistical software Minitab 17. All possible combinations for each group were subjected to a fractional factorial design in the software, which randomly selected dosages that were tested to predict the response of remaining combinations. The

neurotrophic factor dilutions were prepared with the cell culture media matrix (DMEM+10% FBS+1% Antimyotic). After 72 hours, cultures were fixed in methanol, imaged using a widefield microscope with a phase contrast lens, and images captured with a digital camera at 4x magnification. Phase contrast images of DRGs were taken after 3 days of incubation, and the average axonal length was measured.

The data were analyzed using multivariable linear regression model in StataIC 13. The outcome variable was axon length and axon density. Specific doses of a given drug or drug combination were included in the model as indicator variables, rather than assuming a specific form of the dose-response, such as a linear increase across the range of doses. Interaction terms were used to test if the effect of drug combinations were greater than the sum of the effects of individual drugs. Comparisons between specific drug-dose combinations were made by varying the referent drug-dose combination in the included indicator variables, or using Wald post-test comparisons, where either approach produced comparisons analogous to independent sample t-tests. All reported p values are for two-sided comparisons. P values less than or equal to 0.05 were considered significant. In our experiment, the goal was to identify the drug(s)-dose(s) combination that provided the greatest response, or to observe some range of effective doses. Statistical comparisons are provided merely descriptively to indicate where the maximum or plateau of the curve separates from the ineffective drug(s)-dose(s) combinations.
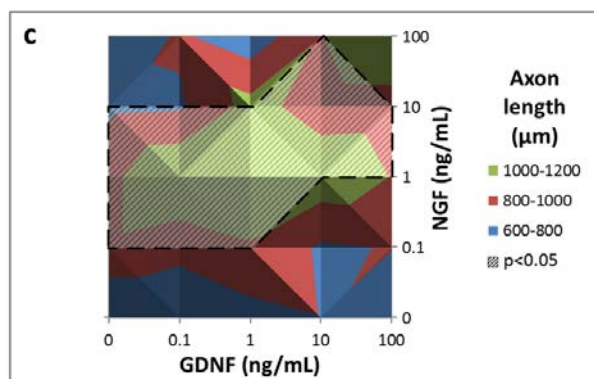
Treatment of the DRGs with NGF concentrations of 0.1ng/mL (910.5 ± 39.8), 1ng/mL (993.7 ± 53.6), 5ng/mL (1063.5 ± 57.8), 10ng/mL (1117.6 ± 55.6) and 100 ng/ml (755.7 ± 57.8) enhanced axonal elongation compared to control 0ng/mL(469.7 ± 23.4; $p<0.05$) (Figure 7). GDNF also promoted axonal growth above that of the control group at concentrations of 0.1ng/mL (706.6 ± 48.3), 1ng/mL (749.3 ± 38.3), 5ng/mL (791.7 ± 65.5), 10 ng/mL (919.6 ± 46.4) and 100 ng/mL (888.0 ± 44.8) ($p<0.05$) (Figure 7).

Figure 7. Plots showing effect of single drug treatments on axonal elongation (left) NGF dosage/length (right) GDNF dosage/length. The bars represent mean±SD (* statistically different from 0 ng/mL; $p<0.05$)
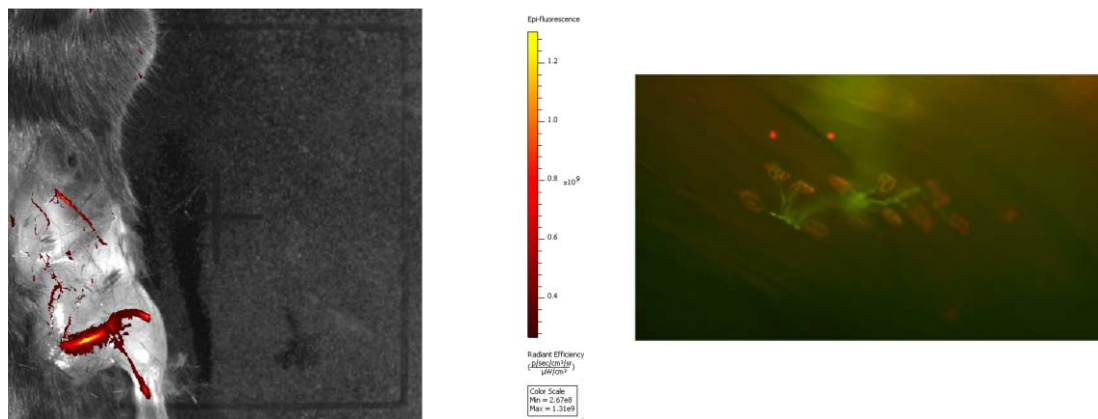
Combined GDNF and NGF worked synergistically and produced significant enhancement of axonal outgrowth as compared to individual treatments, shown in shaded region of Figure 8. The plots suggest a strong effect of increasing NGF concentration (0.1, 1, and 10 ng/ml). The average axonal length at the optimal GDNF+NGF concentrations (1 ng/ml NGF combined with 1 or 0.1 ng/ml GDNF) ($p<0.05$) was 1000–1100 μm, which is higher than the 800–950 μm axonal elongation observed at NGF alone or GDNF alone. Altogether, the results indicated a strong interaction of both growth factors. This interaction was synergistic for axonal growth at all GDNF and NGF concentrations. Statistically, combinations of 1 ng/mL NGF with 0.1 ng/mL, 1 ng/mL or

10 ng/mL of GDNF were found to produce the best axonal growth response (p<0.05 when compared to control).

Figure 8. Surface plots showing effect of combined drug treatments with NGF and GDNF on axonal elongation. (Shaded region specifies the concentration range, which produced significantly different axonal elongation response from 0ng/mL, p<0.05)

3.2.c Aim 3 In Vivo Efficacy: We have acquired the initially breeding pair of mice from The Jackson Laboratories as per our IACUC and ACURO protocol. We have now expanded our breeding colony to include 2 males and 4 females. The average litter size has been 8 pups with all four females having successfully produced offspring. We are currently expanding the colony so that we will have sufficient mice to complete proposed experiments. We have also confirmed by imaging (IVIS Imaging System; 100 series Caliper Life Sciences) and by staining the neuromuscular junction that the mice expressed green fluorescent protein along the length of the nerve and at the neuromuscular junction (Figure 9). We expect to begin the in vivo experiments within the next two months and will have sufficient mice available.

Figure 9. (Left): Fluorescent intensity plot demonstrating that the sciatic, tibial, and peronel nerve all express GFP. (Right). Fluorescent light microscope image of neuromuscular junction with acetylcholine receptors immunohitochemically stained (red; alpha-bungarotoxin) and nerve expressing GFP.

3.3 What opportunities for training and professional development has the project provided?

1. Continuation of PhD research project for Pratima Labroo.
2. Continuation of Surgical Resident research project for Kyle Edwards
3. Continuation of MS research project for Scott Ho.

4. Undergraduate research project for Megan Roach, Rainey Cornaby, and Artemis Sefandonakis

3.4 How were the results disseminated to communities of interest?

Nothing to Report

3.5 What do you plan to do during the next reporting period to accomplish the goals?

3.5.a Specific Aim 1: To optimize release kinetics of NGF and GDNF in vitro using our novel drug delivery conduit.

We have accomplished all the tasks of Specific Aim1.

3.5.b Specific Aim 2*: **To evaluate the effectiveness of the conduit-drug delivery device at releasing bioactive concentrations of NGF and GDNF sufficient to enhance axon growth in dorsal root ganglion (DRG) cell culture**

We have already initiated the dual release of NGF and GDNF from the PTFE device. The release experiments will be finished within the next month. Once we have obtained the release media we will measure the levels of NGF and GDNF with a standard ELISA. We have already optimized ELISA parameters for both assays. Once we know the concentration of NGF and GDNF released from the device we will evaluate bioactivity. We expect both NGF and GDNF to be bioactive as we have already done experiments where we store the growth factors in the cell culture incubator for 30 days as well as released growth factor from the PLGA device, and the growth factors were bioactive. Thus, all techniques have already been conducted in the laboratory and we don't expect any major hurdles to accomplish the remaining Specific Aim 2 tasks.

3.5.c Specific Aim 3: To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a nerve gap in a mouse sciatic nerve model.

We will continue to expand our colony so that we have sufficient mice to conduct the proposed experiments. We have previously preformed all required tasks for this aim: surgeries, walking track, electrophysiology, and histology. Thus, we do not expect any major hurdles to overcome to surgically implant the device and evaluate efficacy.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

4.1 What was the impact on the development of the principal discipline(s) of the project?

One of the key impacts of the present approach is that it is possible to easily manipulate our nerve conduit device design. That is through the use of our mathematical model it is possible to predict what size reservoir and what size hole is required to release a set amount of a drug or growth factor. This was demonstrated by our ability to quickly redesign our device from PLGA to PTFE. Without much troubleshooting we were able to release fluorescently labeled dextran from PTFE, in a manner that was consistent with our model.

4.2 What was the impact on other disciplines?

As part of this work, we have developed a new mathematical model that can be used by researchers to predict reservoir volume, drug amount, drug concentration, and diffusion hole size. This model will help researchers to avoid costly and time intensive in-vitro trials.

We have developed fabrication and sterilization protocols for a nerve conduit device with dual drug reservoirs and tested the efficacy of the device using in-vitro and DRG studies. This data will help researchers/industry to further develop drug delivery efforts in other areas as well.

4.3 What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

4.4 What was the impact on society beyond science and technology?

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

5.1 Changes in approach and reasons for change

Based on data from another project we have switched from PLGA to PTFE. The PLGA did not maintain its structure in vivo. Otherwise the device design is the same with an inner conduit, outer conduit, and diffusion hole. The transition from one material to another was also made easier with our diffusion model. Utilizing the model we were able to accurately predict the appropriate device parameters, such as reservoir size and diffusion hole, required to release NGF and GDNF.

5.2 Actual or anticipated problems or delays and actions or plans to resolve them

We are behind schedule with finishing Specific Aim 2 and Specific aim 3 as a result of changing from PLGA to PTFE. We will be finished with Specific Aim 2 in the next few months. Another advantage of using PTFE is the manufacturing process is considerably quicker than PLGA, such that we can manufacture the device at a rate that is equivalent to the rate at which we can implant the devices in vivo. Thus we can make up a lot of time that we have lost by implanting devices at a quicker rate that initially planned. We have previous experience will all aspects of the animal procedures thus limiting the potential to encounter any major hurdle from that direction.

5.3 Changes that had a significant impact on expenditures

Nothing to report

5.4. Significant changes in use or care of human subjects

Nothing to report

5.5 Significant changes in use or care of vertebrate animals.

Nothing to report

5.6 Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications.
  1. Keng-Min Lin, Jill E. Shea, Bruce K. Gale, Himanshu Sant, Patti Larrabee and Jayant Agarwal. "Nerve growth factor release from a novel PLGA nerve conduit can improve axon growth", submitted to Journal of Micromechanics and Microengineering, acknowledgement of federal support (yes)

  2. Keng-Min Lin, Jill E. Shea, Bruce K. Gale, Himanshu Sant, Srinivas Chennamaneni, Michael Burr and Jay Agarwal. PDMS drug delivery devices: potential application in nerve regeneration, Biomedical Microdevices, in preparation, acknowledgement of federal support (yes)

  Books or other non-periodical, one-time publications.

  Keng-Min Lin, IMPLANTABLE DEVICES FOR SENSING AND DRUG DELIVERY IN OPHTHALMOLOGY AND RECONSTRUCTIVE SURGERY, Ph. D. Dissertation, Department of Mechanical Engineering, University of Utah, May 2014, acknowledgement of federal support (yes)

  Other publications, conference papers, and presentations.

  Scott Ho, Pratima Labroo, Keng-Min Lin, Himanshu Sant, Jill Shea, Jay Agarwal, Bruce Gale, Bioresorbable Multi-Drug Delivery Conduit to Promote Peripheral Nerve Regeneration, in Proceedings of 2014 BMES Annual Meeting, San Antonio, Texas, October 22-25, 2014.

  Pratima Labroo, Jill E Shea, Himanshu Sant, Bruce Gale, and Jayant Agarwal, Controlled Delivery of Growth Factors and Small Molecules for Peripheral Nerve Regeneration, oral presentation at 2015 AIChE Annual Meeting November 10, 2015.

- **Website(s) or other Internet site(s)**
-
  http://www.mems.utah.edu/publications/
  This website lists the publications and research originating from Co-PI Dr. Gale's lab.

- **Technologies or techniques**

Fabrication of biodegradable drug delivery prototypes using PLGA. We will publish journal articles to share the device fabrication techniques.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  1. SANT HIMANSHU JAYANT, GALE BRUCE KENT, AGARWAL JAYANT P, LIN KENG-MIN, METHODS AND DEVICES FOR CONNECTING NERVES, Last status change:2013-05-10/ Fill date:2012-10-16, WO 2013066619

- **Other Products**
  1. Mathematical model based on Fick's diffusion law
  2. Fabrication of dual chamber combined PLGA nerve guide and drug delivery device prototypes
  3. Use of laser to create diffusion hole

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Personnel	Role	Percent Effort	Months
Jay Agarwal	PD/PI	8.33	1.0
Overall management of the project, guidance to students, weekly meetings and report preparation.			
Bruce Gale	Co-I	7.83	1.0
Device manufacturing, weekly meetings			
Jill Shea	Co-I	16.5	2.0
IACUC/ACURO approvals, DRG studies, ELISA, histology, animal studies, weekly meetings.			
Himanshu Sant	Co-I	21.16	2.5
Device manufacturing and validation, mathematical model, weekly meetings and report preparation.			
Christi Terry	Co-I	13.5	1.5
DRG studies, ELISA optimization			
Gregory Clark	Co-I	5.25	1.0
Electrophysiology protocol preparation			
Kyle Edwards	General Surgery Resident	50.00	6.0
DRG cell culture, ELISA, animal procedures, weekly meetings			

PLGA dual chamber prototype fabrication, mathematical model, weekly meetings.			
Pratima Labroo	Student	25.00	4.0
DRG studies, ELISA, animal studies, and weekly meetings.			

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

None

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

None

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Attached

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

A Novel Drug Delivery Device for Peripheral Nerve Regeneration

OR120181
W81XWH-13-1-0363

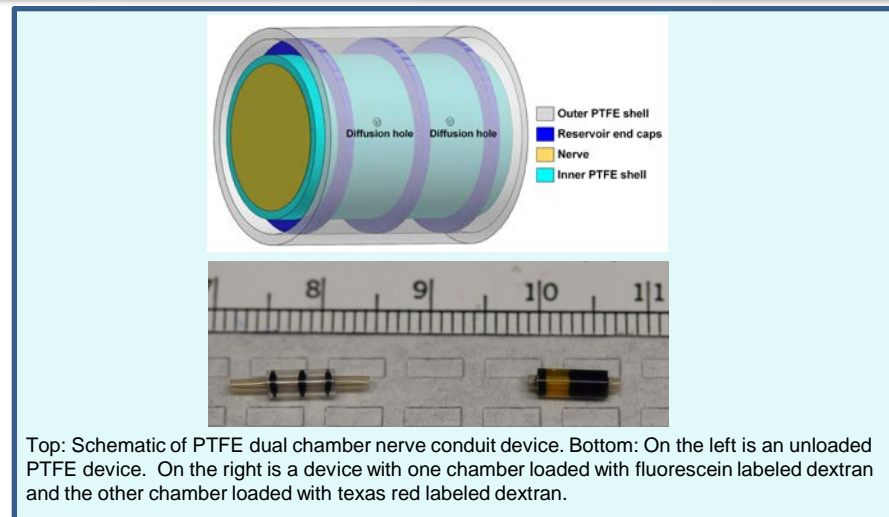**PI:** Jayant Agarwal   **Org:** University of Utah   **Award Amount: Direct** $499,972; **Total** $744,958

Study Aims

• To optimize release kinetics of NGF and GDNF in vitro using our novel drug delivery conduit.
• To evaluate the effectiveness of the conduit-drug delivery device at releasing bioactive concentrations of NGF and GDNF sufficient to enhance axon growth in Dorsal Root Ganglion (DRG) cell culture.
• To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a nerve gap in a mouse sciatic nerve model.

Approach

This proposal will the efficacy of a polytetrafluoroethylene (PTFE) nerve guide to improve nerve regeneration. Specifically, this project explores the potential of combing a nerve conduit that will act as an axon guide for the regenerating nerve and a drug delivery device to deliver nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) for at least 30 days to improve rate of nerve regeneration

Top: Schematic of PTFE dual chamber nerve conduit device. Bottom: On the left is an unloaded PTFE device. On the right is a device with one chamber loaded with fluorescein labeled dextran and the other chamber loaded with texas red labeled dextran.

Accomplishment: We can manufacture the dual chamber PTFE devices and are in the process of evaluating NGF and GDNF release bioactivity.

Timeline and Cost

	Activities CY	Year 1	Year 2	Year 3
Aim 1	Mfg. of the drug delivery device-nerve guide conduit prototypes COMPLETED			
	Optimization of diffusion hole COMPLETED			
	Optimization of reservoir dimensions COMPLETED			
Aim 2	In Vitro Release Kinetics PARTIALLY COMPLETED.			
	EDRG axonal growth PARTIALLY COMPLETED			
	Data analysis			
Aim 3	Animal testing of novel drug delivery device			
	Data analysis			
	Report			
Estimated Budget ($)		242,766.00	247,246.00	254,946.00

Goals/Milestones

CY Year 1 Goal – **Optimize release kinetics of NGF & GDNF** *in vitro*
☑Prototype fabrication and reservoir optimization: conduit dimensions
☑ Diffusion hole optimization: porosity, pore size and dimensions
☑ Optimization of NGF & GDNF
CY Year 2 Goal – **Determine growth factor bioactivity with Dorsal Root Ganglion (DRG) cell culture**
☐Testing with growth factors at varying concentrations, media control and fresh aliquots for NGF and GDNF separately and in concert.
CY Year 3 Goal – Determine device efficacy with mouse sciatic nerve
☐Implant drug delivery device and compare with autologous nerve and empty nerve conduit
☐Complete walking track, histological analysis, electrophysiology and immunohistochemistry
Comments/Challenges/Issues/Concerns None
Budget Expenditure to Date
Projected Expenditure: $247,246.00 FOR THE YEAR
Actual Expenditure: *: $ 226,796.91 ($152,212.62 Direct + $74,584.299 Indirect)* for Year two ending October 1, 2015

Updated: October 26, 2015